Instruction Manual

Semi-dry Blotting System
WSE-4045
HorizeBLOT 4M

Ver. 2
November. 29, 2018

Made in Tokyo
ATTO
Supporting Life Research

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Introduction

Thank you for purchasing WSE-4045 HorizeBLOT 4M, an ATTO scientific instrument. The product is delivered to you along with the device manual to support full utilization of the product in your laboratory. Not only first-time users of the product but also experienced users are asked to read an understand thoroughly this manual before use.

Before using the device for the first time, please read this manual from beginning to end. Furthermore, this manual contains instructions and information on maintenance, the warranty, and service, in addition to the operation. Keep this manual at hand for effective use.

Feel free to contact us for enquiries about your purchased product or the manual.

Instruction manual

Please read this manual thoroughly to become acquainted with the proper use of the product. After reading, keep this manual readily available for reference. If the product is to be relocated, make sure to include this manual with the product.

If missing pages or binding errors are found, lost or damaged, a new copy will be provided. Please contact your sales agency or us and give the product name and model name. Every effort has been made to prepare this manual accurately and completely, but if any points of query, errors, or omissions are found, please notify us.

Safety precautions

Proper operation of the device assures your safety. Please read this manual thoroughly to become acquainted with operation of the device prior to its use. The operation procedures and safety precautions in this manual refer to the use of the device for its intended purposes. Do not operate the device for purposes other than those intended or in a manner other than that described in this manual. If the device is used for purposes other than those intended and in a manner other than that described in this manual, the person who operates the device shall be responsible for necessary safety measures.

Safety symbols

The following symbols are used in this manual and on the product to assure and maintain safety in the use of the device. Please familiarize yourself with the meaning of these symbols and always observe the instructions that follow these symbols.

Symbol	Description
 Danger	Failure to observe this precaution could lead to an imminent risk of death or serious injury.
 Warning	Failure to observe this precaution could lead to risk of death or injury.
 Caution	Failure to observe this precaution could lead to the damage of property.
	This symbol indicates prohibited acts.
	This symbol indicates important information.
	This symbol indicates tips and hint.

Operation precautions

These precautions are necessary to prevent fire, electric shock, or other accidents and failures.

Danger

Power connection 	Do not use this type of power cable, of which terminals and plugs are deformed or corroded, or of which insulation cover is separated or damaged. It causes electrical shock or fire due to poor contact. Refrain from using the device and contact us. After turning off the device, disconnect the power cable from the receptacle. On unplugging the power cable, hold the plug, but do not pull on the cable.
No wet hand 	Do not operate the device with wet hands. Also do not touch the power plugs and terminals with wet hands. It causes electrical shock or failure of the device.
Main body 	Do not insert a foreign material into the device. It causes electrical shock or failure of the device. Do not use the device in wet condition. These causes electrical shock or failure. Clean all moistures up from surface of the device sufficiently when you use it.
Maintenance 	Please quit using the device immediately if anything abnormal happens during device usage or if any abnormality/failure is suspected. Also, do not use the device if you find any failure at inspection. These can cause electrical shocks or damage to the device. During the operation with blotter, please check if there is any abnormal sound or leaking or visually suspected troubles. Contact us if you detect any abnormality, trouble or failure.
Reagents 	In operation of electrophoresis or blotting, it may use substances with deleterious substance, hazardous materials, and carcinogenic for Preparation of buffer solution, staining or bleaching operation. Never contact it with human body directly, it causes accident or failure to the human body to cause death or burns. In use of chemicals, carefully read the handling cautions or warning of chemicals and protect your body with gloves and masks.

Warning

Installation location 	Please avoid installation on places such as a wobbling table, tilted location, or heavily vibrating place. Install the Unit on horizontal places with safe and hard surfaces, such as a lab bench. It causes electrical shock failure by leaking liquid or falling down. Do not put anything on the device, it causes electrical shock by falling down.
Main body 	This device is not an explosion-proof structure. Install this device away from any places with the possibility of exposure to fire or flammable gasses. When the device is moved from a low-temperature area to a high-temperature area, there is possibility of [Condensation], i.e. moisture in the air becomes water drops. If condensation happens within the device, get it dry sufficiently. It causes electrical shock or failure.
Power connection 	Please set the maximum voltage value 150V and the maximum current value 3A when this device is connected with power supply. If used in maximum setting value over 150V or 3A, this can cause damage to the device.
Migration 	Never move the device or touch the operating panels of power supply during operation. It causes electrical shock by flowing out of liquid. Cords may get entangled, which might cause rollover. Always turn off the power switch and disconnect the AC cord or lead wire when you move this device.

Warning

Maintenance 	Please be sure to turn the Unit power switch OFF and pull out the power cable before performing maintenance and cleaning. These might cause electrical shock. Please ask us for periodical maintenance, inspection and calibration so as to maintain good performance and safety of the device.
Disassemble Prohibition 	Never disassemble or modify the device. Adjustment and repair work of the product should be done by our service staff in charge. When adjustment or repair is required, please ask our company. Any accident caused by your disassembling or modification would be outside of our responsibility.
Seal Group 	Never peel off the Warning Seal. The seals indicate dangerous areas. Please contact us if any seal peeled off or dirty and cannot be read.

Caution

Seal Group	Product name seals are important information for maintenance and managing products. Don't peel it off. Please do not remove or stain the seal.
Safety lead	The safety lead of this device is exclusive use, do not use it except for blotting. It may cause accident or failure. Any accident caused by improper using of the power lead would be outside of our responsibility. If you have any questions, inquire our sales agent or company.

Other precautions

Application	WSE-4045 HorizeBLOT 4M is a laboratory equipment, not for medical use. Never use this device for diagnostic or medical purposes.
Export	Export of specific work and cargo are controlled by Foreign Exchange Laws and Cabinet Order/Ministerial Orders of Foreign Trade Control Laws and those controls are applied to this Unit. Even if the Unit is not applicable to the Cabinet Order, it is required to submit documents accordingly and if it is applicable, then obtain export license from the Ministry of Economy, Trade and Industry, and then submit the license to the customs office. When you export our product, please confirm with your supplier or our customer service department in advance.
Trademark・Copyright	Copyright approval is required when you make copy/reprint a part or whole of the Instruction Manual. Specifications of the Unit and contents of the Instruction Manual may be changed without an advanced notice.

1 Overview

1.1 Purpose of use

HorizeBLOT 4M-R is used to transfer samples in a gel onto a membrane. Polyacrylamide gels of compact size (60 x 60mm), mini size(90 x 80mm), and wide size (140 x 80mm) can be applied.

2 Confirmation when unpacking

2.1 Confirmation when unpacking

Upon arrival of the product, please confirm whether the main body and accessories are packed correctly or if any defects exist.
In case there is any inadequacy, deficiency, etc., please inform your supplier or our company immediately. Please perform your confirmation as above at the time of unpacking within one week upon arrival of the Unit. Confirmation after more than one week may result in not receiving compensation for defects or missing articles.

2.2 Equipment configuration

This products consists of a main body and accessories.

Main body

Main body : Semi-dry blotting system, Model : WSE-4045 HorizeBLOT 4M

Model	WSE-4045	WSE-4045・M
Code No.	2322476	2322477
Main body	HorizeBLOT 4M	
Membrane	-	WSE-4051 ClearBlot P plus membrane 85×90mm, 20/pk
Filter paper	-	CB-09A Absorbent Paper 85×90mm, 400/pk

Materials

Anode plate	Platinized titanium
Cathode plate	Corrosion-resistant stainless steel
Base	Acrylic
Cover	Acrylic

Accessories

Model	WSE-4045	WSE-4045・M
Safety lead	1	
Roller	1	
Instruction manual	1	

3 Name and function of each parts

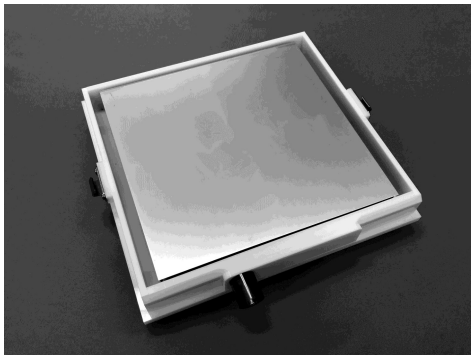
3.1 Main body

This product consists of a (1) cover and (2) base.

A condition in which the cover and the base are combined

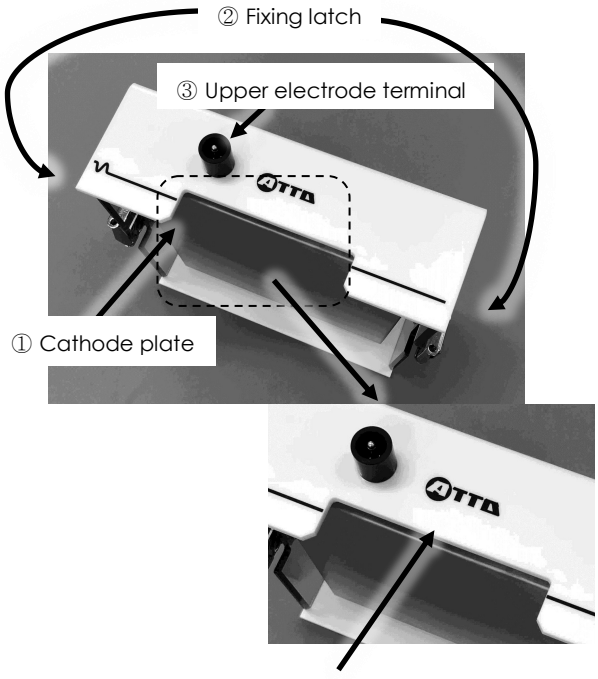
(1) Cover

(2) Base



(1) Cover

Included cathode plate, using the base unit together.
Connect to the cathode (-) of the power supply.



① Cathode plate

A corrosion-resistant stainless steel electrode plate which becomes a cathode electrode (-) by connecting to the cathode terminal of the power supply.

② Fixing latch

Fix the cover and the base with the bracket on the base.

③ Upper (cathode) electrode terminal (black)

Connect to the safety lead.

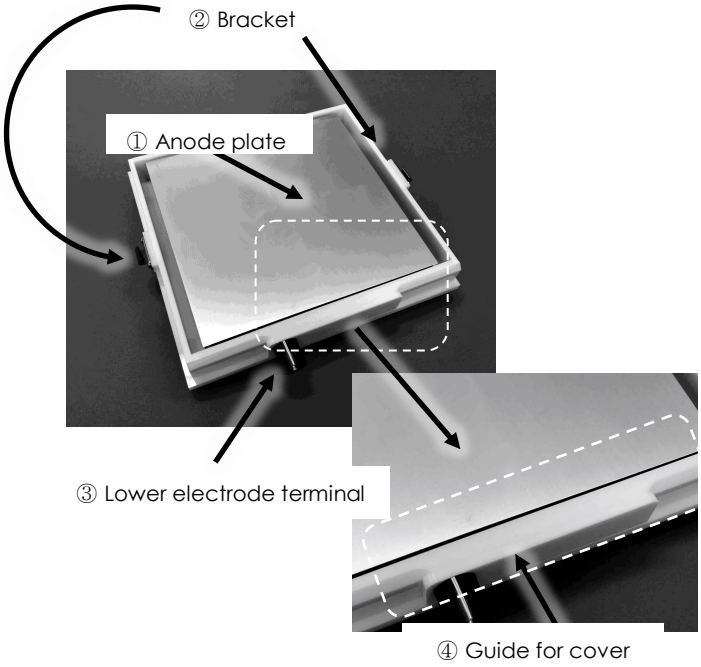
④ Guide for base

Using for the adjustment of aligning of the cover and the base.

(2) Base

Included anode plate, using the cover unit together.

Connect to the anode (+) of the power supply.



① Anode plate

A platinized titanium electrode plate which becomes an anode (+) by connecting to the anode terminal of the power supply.

② Bracket

Using the fixing latch and the bracket, fix the cover and the base.

③ Lower (anode) electrode terminal (red)

Connect to the safety lead.

④ Guide for cover

Using for the adjustment of aligning of the cover and the base.

3.2 Accessories

Safety lead

A safety lead including function of protection against a reverse connection. Anode (+) terminal connected with Red lead wire. Cathode(-) terminal connected with Black lead wire.

Roller

Use to remove air bubbles and excessive blotting buffer between membrane, paper and gels.

4 Preparations

4.1 Usage environment

This apparatus should be used in the environment described below.

Location	For indoor use only
Operating temperature/humidity	5~40℃・5~70%RH（without condensation）

Warning

This apparatus should not be placed in an environment where there is inflammable gas. There is a possibility that an explosion and/or fire could occur because it does not have an explosion-proof structure. Please install it in an environment where there is no contact with inflammable gas.
This apparatus should not be placed in an environment where there is corrosive gas that may cause a corrosion of conductors inside the apparatus and poor contact between connectors, resulting in mechanical errors and/or failure, or fire.
This apparatus should not be placed in a dusty environment where dust or other dirt may collect on this apparatus, because it may cause electrical shocks, fire, or mechanical failures.

Caution

This apparatus should not be used in a place where there is a strong magnetic or electric field, or excessive source-voltage fluctuation and/or electrical noise. It can be a cause of mechanical errors.
This apparatus should not be placed in a location where there is direct sunlight, or where abrupt temperature changes or high humidity occur. Do not use this apparatus when condensation has occurred.
This apparatus cannot be used outdoor. It is designed to ensure safety and performance under conditions of an ambient temperature of 4℃ to 40℃ and relative humidity of 5 to 70% (no condensation).

4.2 Preparation of experimental equipment

Power supply
Suitable for blotting that can output high current.
Voltage ~150 V, Current ~3.0 A

Ex. 1 ATTO WSE-3100 PowerStation Ghibli I
ATTO WSE-3500 PowerStation HC

When gel size is small, it can be used with the following specifications.
(1 ATTO Mini gel, 1 ATTO Compact gel)
Voltage ~150 V, Current ~500 mA

Ex. 2 ATTO WSE-3200 PowerStation III
ATTO AE-8450 PowerStation 1000VC (Old ver.)

Shaking apparatus
Used during staining or destaining, and the reaction of the gel and the blotted membrane.

ATTO WSC-2400 Seesqw Shaker ATTO

Imaging System
Used for detecting colorimetry and luminescence of gel and blotted membrane.
ATTO Printgraph series (Colorimetry)
ATTO LuminoGraph series (Luminescence)

4.3 Preparation of consumables

Prepare membrane and filter paper to fit the gel size.

	Membrane	Filter paper	Clear pocket for membrane seal
Compact gel (60x60x0.75mm)	WSE-4050 ClearBlot P plus membrane (65×65mm)	CB-06A Absorbent paper (65×65mm)	Pitatt Clear
Mini gel (85x90x1mm)	WSE-4051 ClearBlot P plus membrane (85×90mm)	CB-09A Absorbent paper (85×90mm)	
Wide gel (85x150x1mm)	WSE-4053 *1 ClearBlot P plus membrane (260mm×3m)	CB-20A *1 Absorbent paper (200×200mm)	

*1. For wide gel (140x80mm), cut the membrane (WSE-4053) and filter paper (CB-20A) to 150x85mm.

※The number of filter paper varies depending on the blotting buffer.
The number of filter paper used for out blotting buffer is shown in the table below.

Blotting buffer	Number of filter paper
AE-1460 EzBlot	3 sheets each of cathode and anode
AE-1465 EzFastBlot	2~3 sheets each of cathode and anode
WSE-7210 EzFastBlot HMW	3 sheets each of cathode and anode
WSE-4055 QBlot kit	-

※The number of filter paper is based on ATTO 0.9 mm thickness product. When using filter paper of different thickness, refer to the above number, please adjust to the same thickness.

4.4 Preparation of reagents

1. Required reagent

Depending on the purpose, prepare the following reagents.

1) Blotting buffer
2) Primary antibody and labeled secondary antibody
3) Blocking reagent
4) Wash buffer
5) Detection reagent
6) Reprobing reagent

ATTO sells the products described in 「7.4. Consumables and reagents」 on page 25. Please use it according to the application.
For details of the product, please contact us.

2. Preparation of reagents

Please refer to the instructions and references for each reagent you use.
The following is a brief explanation of the preparation of reagents for use mainly with our related products.
Please refer to the instruction manual attached to each product for more information.

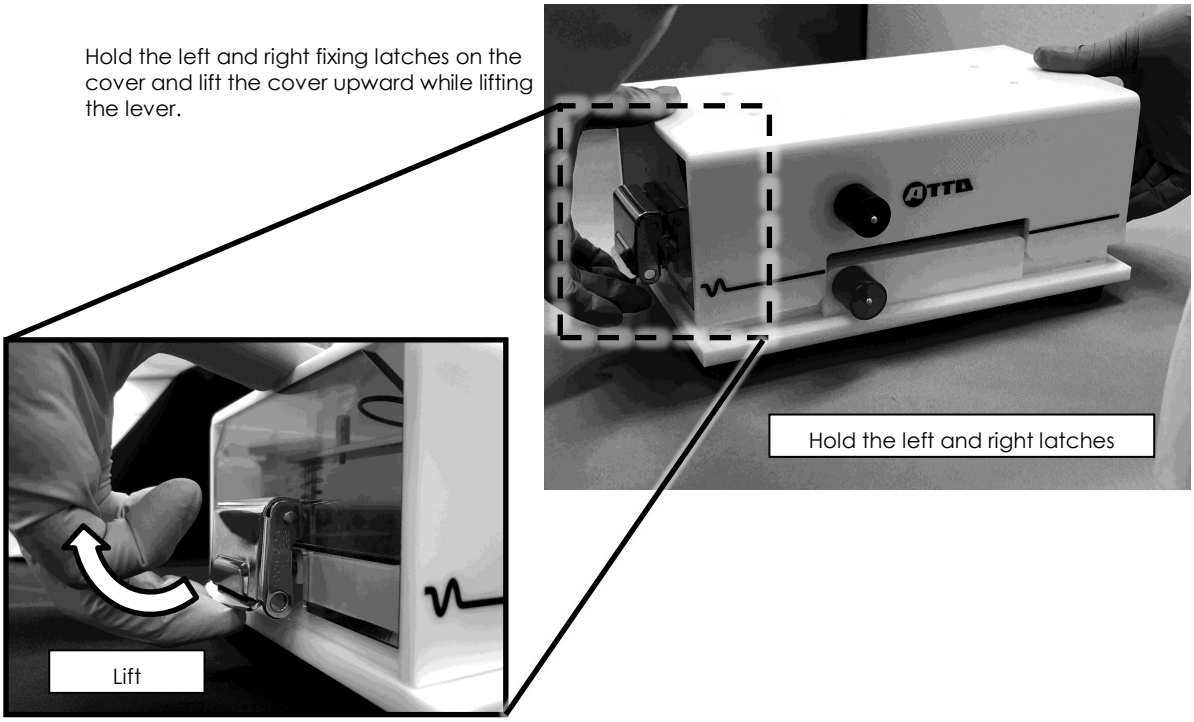
	Model	Product name	Preparation	Quantity (for 1 mini gel)
Pre-wet PVDF membrane and filter paper	WSE-4055	QBlot kit	Dilute Gel Wash Buffer to 1/5 with distilled water.	50mL
Blotting buffer	AE-1460	EzBlot	Add 25 mL of methanol to unopened A~C bottles, respectively.	About 50 mL each of A~C
	AE-1465	EzFastBlot	Dilute to 1/10 with distilled water.	About 200 mL
	WSE-7210	EzFastBlot HMW	Dilute to 1/5 with distilled water.	
	WSE-7055	EzRun TG	Dilute to 1/10 with distilled water. Add methanol to 5~20% according to purpose.	About 200 mL
Blocking buffer	AE-1475	EzBlock Chemi	Dilute to 1/5 with distilled water. AE-1476/77: Add 1/100 amount of Tween 20 included.	About 50 mL (Prepare 650 µL of diluted solution/1 $cm^{2.}$)
	AE-1476	EzBlock BSA		
	AE-1477	EzBlock CAS		
Wash buffer	WSE-7230	EzTBS	Dilute to 1/10 with distilled water. If necessary, add a surfactant such as Tween 20 or Triton X-100 to a final concentration of 0.02 to 0.5%.	Prepare about 300 mL of diluted solution.
	WSE-7430	EzPBS(-)		
Detection reagent (Colorimetry)	AE-1490	EzWestBlue	Use undiluted solution as it is.	About 10 mL (About 100 µL/1 cm^2 membrane.)
Detection reagent (Luminescence)	WSE-7110	EzWestLumiOne		About 5 mL
	WSE-7120	EzWestLumi Plus	Reagent A and B are mixed 1:1 immediately before detection.	Prepare 2.5 mL each for A and B, respectively.
Reprobing reagent	WSE-7240	EzReprobe	Use undiluted solution as it is. If necessary, add 0.6 g of 「Enhancer」 to 100 mL.	About 30 mL Also prepare a wash buffer.

5 Operation

5.1 Preparation of the apparatus

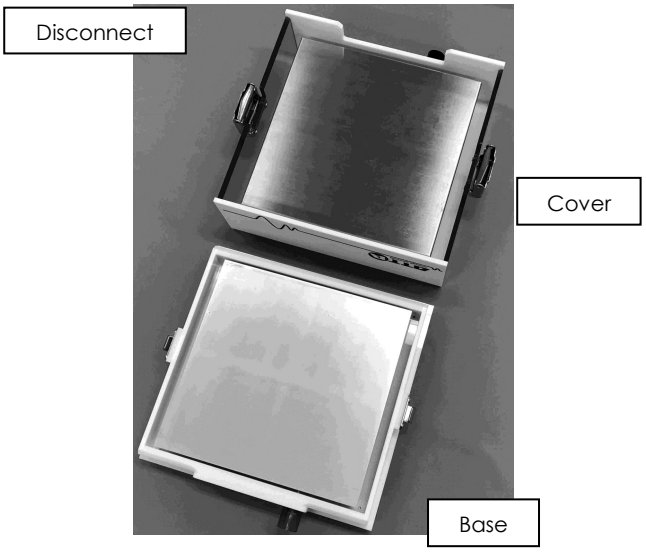
As shown in the figure on the right, when the cover and base is united, remove the cover as follows.

Hold the left and right fixing latches on the cover and lift the cover upward while lifting the lever.



Remove the cover from the base.

From now on, operate the apparatus with the cover removed from the base until the setting of filter paper, membrane and gel is completed.



5.2 Pretreatment

Prepare containers for pretreatment of membranes, filter paper and gel, respectively.

1. Pretreatment of membrane (Hydrophilization)

Soak ClearBlot P plus Membrane in 100% methanol at the bottom and to saturate it for approximately 20 sec. Then immerse this membrane in buffer and saturate it for 5 to over 10 min.

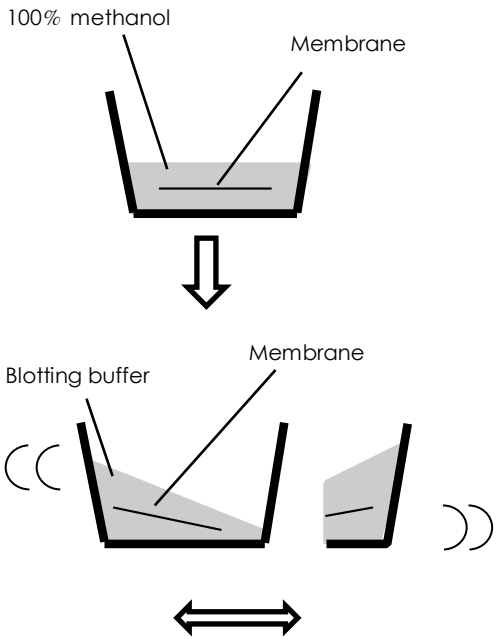
※Never touch the membrane with the bare hands. Handle it only with hands covered in clean experimental gloves or with tweezers.

※This should be prepared before the completion of the gel electrophoresis.

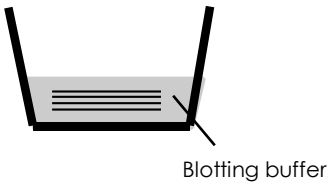
※Since the membrane tends to repel blotting buffer at first, make sure the membrane is saturated with buffer by shaking the container rather strongly. Insufficient saturation of the membrane may cause uneven blotting.

※Please use the membrane with the same size as the gel.

1. Pretreatment of membrane



2. Pretreatment of filter paper



2. Pretreatment of filter paper

Prepare a required number of Absorbent paper (filter paper). Soak all filter papers in blotting buffer for more than 10 sec.

※Prepare before gel electrophoresis is completed.

※Please use a sufficient volume of blotting buffer so that the filter paper is completely soaked in the blotting buffer.

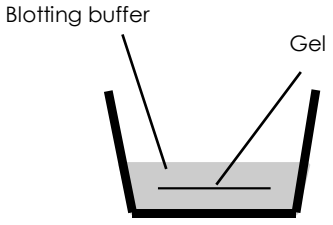
※When immersion for a long time, the filter paper may disintegrate.

When other filter paper than ATTO Absorbent Paper is used, use filter paper of 0.9 mm thickness, or adjust the number of papers used to achieve the same thickness (page 8), and cut them to be the same size as the gel. Filter paper of a different thickness and size from those of ATTO Absorbent Paper may cause uneven blotting, or it may not be possible to perform blotting itself if the power source used has a protection circuit.

3. Pretreatment of gel

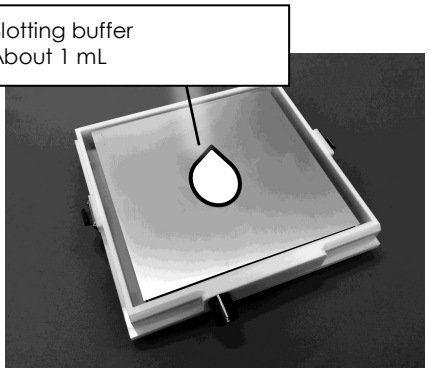
After electrophoresis is completed, remove the gel from the glass plate. Remove extra gels such as the lower part of the gel and the sample well part of the gel. Then rinse with a blotting buffer and remove the electrophoresis buffer and gel pieces adhering to the gel. If left for a long time (more than 5 min), the gel may swell or low molecular weight proteins may get out of the gel, and blotting efficiency may decrease. In addition, uneven blotting or 「Err」 is displayed on power supply unit, and blotting may not be possible.

3. Pretreatment of gel



5.3 Setting of filter paper-membrane-gel

Drop approximately 1 mL of blotting buffer on the anode plate.

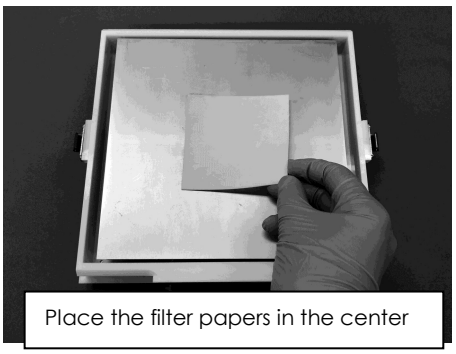


During the operations, make sure to wear clean experimental gloves on both hands.

Remove the filter papers that have been soaked in blotting buffer and drain them.

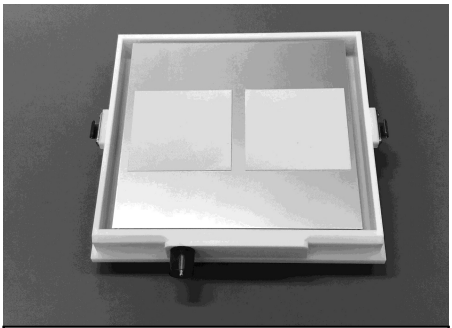
Whenever filter paper is removed from the buffer, perform the same procedure as described above.

For a single gel, place the filter papers in the center of the anode plate.



When blotting only one gel, ensure that the filter paper is placed at the center of the anode plate. If the filter paper is placed closer to the end of the anode plate, may cause uneven blotting or 「Err」 is displayed on power supply unit, and blotting may not be possible.

When blotting more than one gel, ensure that the filter paper is placed on the anode plate evenly. If the filter paper is placed closer to the end of the anode plate, may cause uneven blotting.



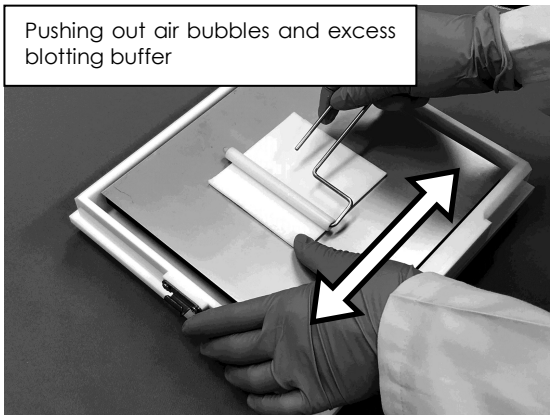
Place the membrane soaked in the blotting buffer on the filter paper to match the four sides of the filter paper.

※ Please pay attention to prevent trapping air bubbles between the filter paper and the membrane.

Gently press one side of the membrane with a finger to avoid moving the filter paper and the membrane. Pushing out air bubbles and excess blotting buffer by rolling a roller from one side of the membrane where the finger is pressed toward the other side.

Repeat rolling the roller in the opposite direction.

※Excessive blotting buffer will exude from the paper, but it's not a problem.



Follow the instructions above not to trap air bubbles, blotting would be obstructed in the part where air bubbles remain.

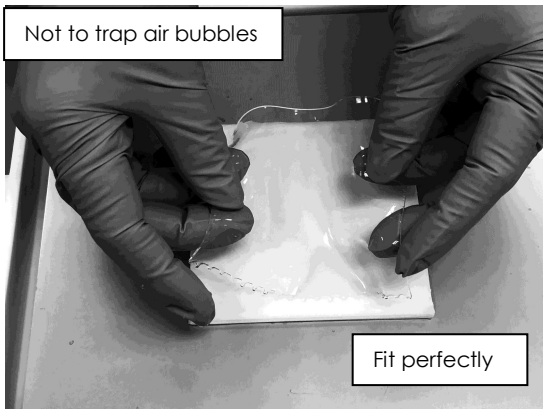
Drop approximately 1 mL of blotting buffer on the membrane.

Place the gel rinsed with blotting buffer on the membrane.

※Place the entire gel is contained within the four sides of the membrane.

※Stack the gel on the membrane

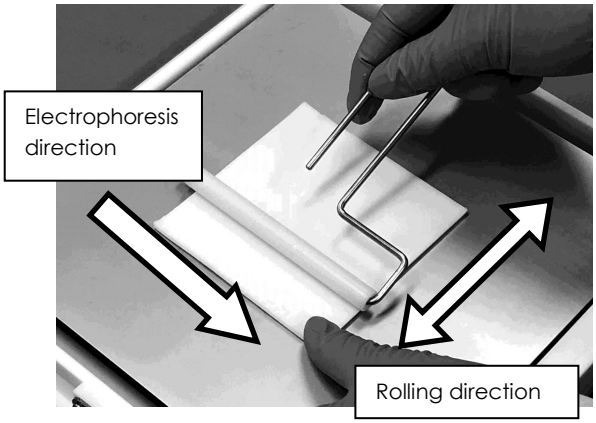
※Do not re-place the gel on the membrane. The sample may transfer on the membrane by just contact with gel and membrane.



Gently press one side of the membrane with a finger to avoid moving the filter paper and the membrane. Pushing out air bubbles and excess blotting buffer by rolling a roller from one side of the membrane where the finger is pressed toward the other side.

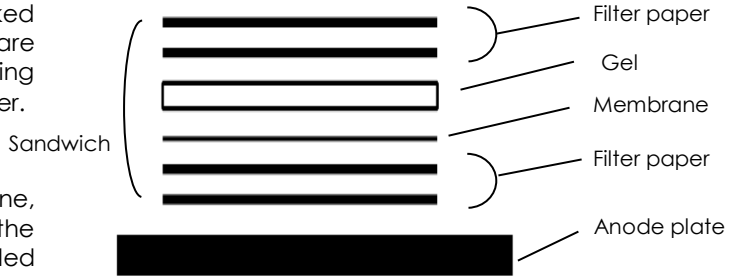
Roll the roller at right angle to the electrophoresis direction.

※Roll the roller gently. With strong forces, the gel may shift or break.



Stack filter papers that have been soaked in blotting buffer on the gel, taking care that air bubbles are not trapped, fitting the four sides of the gel to the filter paper.

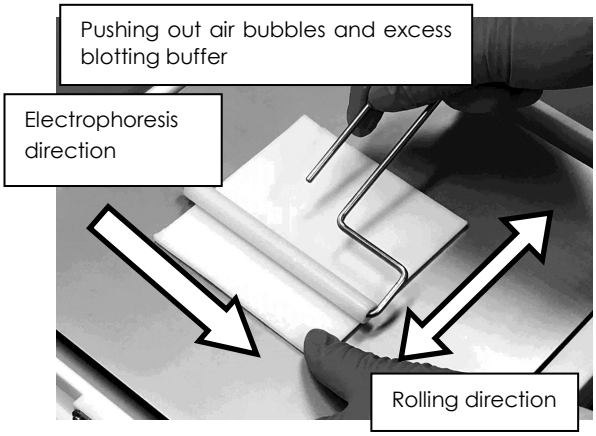
Now the sheets of filter paper, membrane, and gel are stacked as shown on the right. This structure will be called 「sandwich」 hereafter.



Gently press one side of the filter paper with a finger to avoid shifting the sandwich. Pushing out air bubbles and excess blotting buffer by rolling a roller from one side of the filter paper where the finger is pressed toward the other side.

Roll the roller at right angle to the electrophoresis direction as shown on the right.

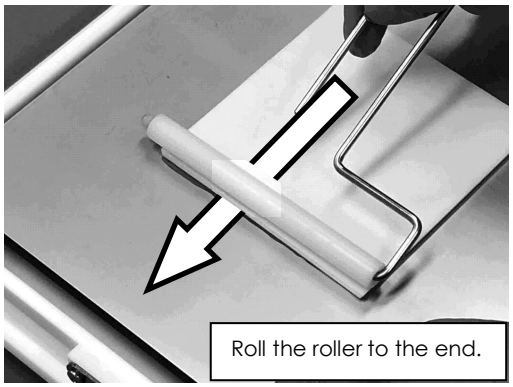
Repeat rolling the roller in the opposite direction.



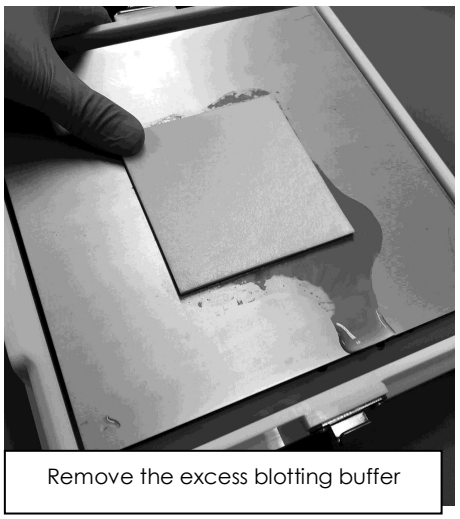
※Excessive blotting buffer will exude from the paper, but it's not a problem.

※Weak contact between the gel and the membrane may cause an obscure blotting pattern.

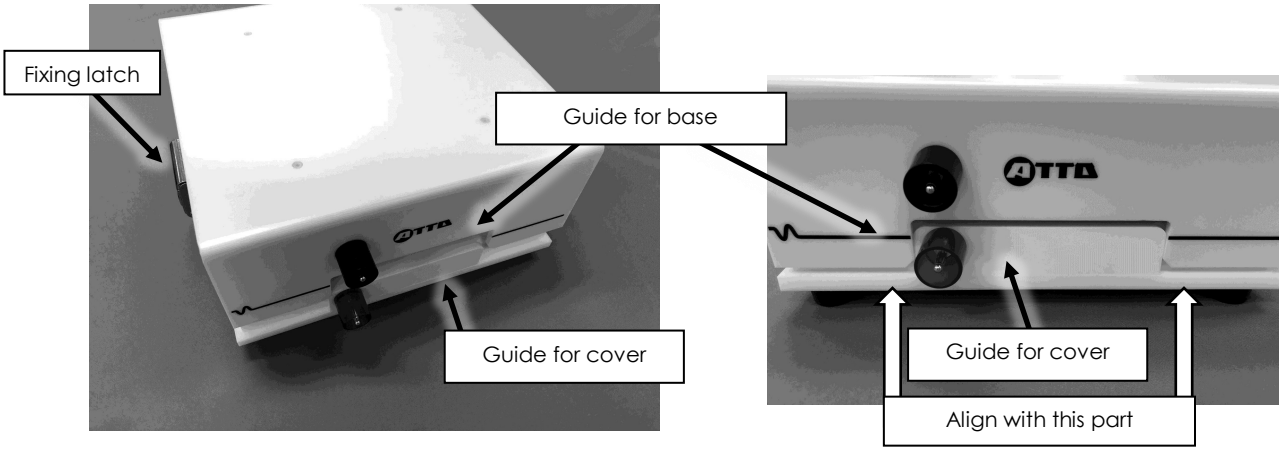
※Do not stop the roller at the edges of the sandwich, roll the roller to the end.



Tilt the electrode plate to remove excess blotting buffers on the electrode plate.

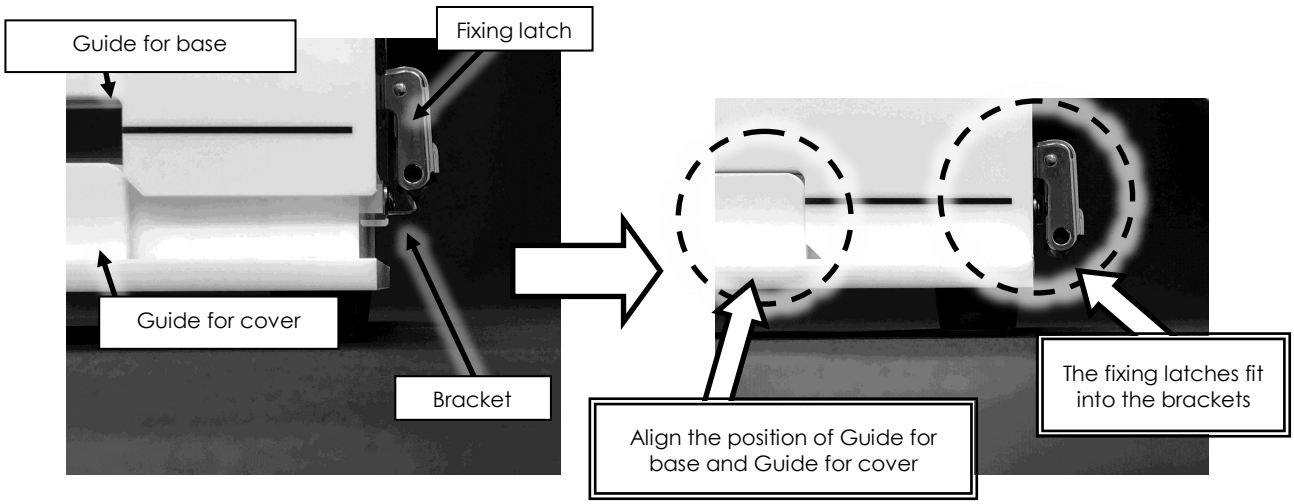


Lift the cover by holding the left and right fixing latches of the removed cover. Pull up the lever of the fixing latches and align the「Guide for base」of the cover with the「Guide for cover」of the base. Fit the cover to the base.



When the「Guide for base」of the cover and the「Guide for cover」of the base fit perfectly, release the fixing latch lever.

The fixing latches fit into the brackets to lock the cover in place.



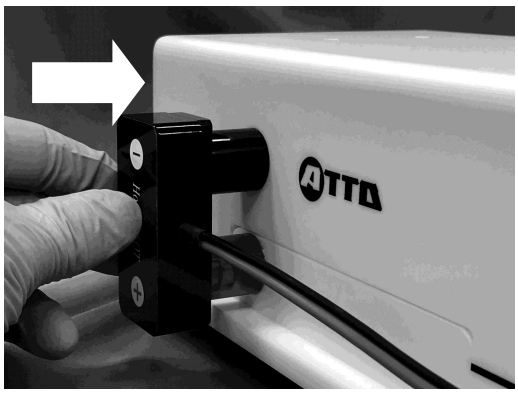
Do not disassemble the cover until the transfer is finished. Once the gel and the membrane contact, the sample may transfer to the membrane. Lifting up the cover may cause displacement between the gel and the membrane which may cause the results to be disordered.

5.4 Connection of the power supply

Warning Confirm that the power supply is turned off and all LED lights are off before connecting the lead to the power supply.

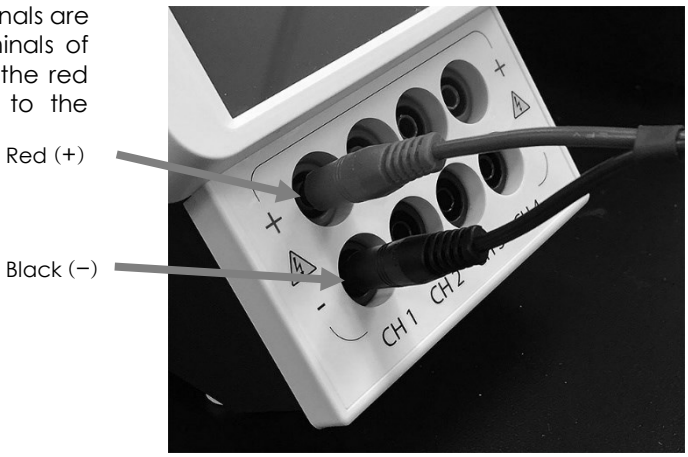
Insert a safety terminal into the electrode terminal of the main body, while aligning the position of the +/- indicators on the main body and the lead assembly (the cathode (black) is above and the anode (red) is below)

The safety lead (for R) with a safety terminal included in the main body should be used. If it is not used, protection against a reverse connection will not work.



Caution Do not use any other lead than the lead assembly (for R) with an included safety terminal for this apparatus. The electrode plate may be destroyed if the cathode and the anode are reversely connected and the power is turned on.

Connect the lead assembly to the power supply. Positive and negative terminals are inserted into the connection terminals of the power supply (the red line to the red (+) terminal and the black line to the black (-) terminal).



5.5 Power supply

Set the output conditions of the power supply. Set values by referring to the information in the table below. In WSE-4045, control under constant voltage output condition is recommended.

［Reference］

When using a hand-made mini-slab gel (approximately 8 x 9 cm) for blotting, the applied current will be 450 to 500 mA at a constant output voltage of 24 V for 10 to 20 min.

Since the setting conditions and conduction time depend on the concentration of the gel, and the types of applied sample, etc., determine more appropriate conditions for your samples with a preliminary experiment using examples from the conditions listed in the table below.

［Reference］

In case of poor blotting efficiency, extend the conduction time.

Do not increase the voltage value. Generated heat may increase and filter paper or membrane may dry.

Blotting buffer	Gel	Number of filter paper	Output conditions and time	
			12V	24V
AE-1460 EzBlot	Hand-made gel	3 sheets each of cathode and anode	30~60 min ~300 mA/gel	-
	ATTO precast gel			
AE-1465 EzFastBlot	Hand-made gel	2 sheets each of cathode and anode	20~60 min ~300 mA/gel	10~25 min ~500 mA/gel
	ATTO precast gel			5~15 min ~500 mA/gel
WSE-7210 EzFastBlot HMW	Hand-made gel	3 sheets each of cathode and anode	30~60 min ~300 mA/gel	15~30 min ~500 mA/gel
	ATTO precast gel			
WSE-4055 QBlot kit	Hand-made gel	1 set each of cathode and anode	15~30 min ~0.6 A/gel	5~10 min ~1.6 A/gel
	ATTO precast gel			

※The number of filter paper is based on ATTO 0.9 mm thickness product. When using filter paper of different thickness, refer to the above number, please adjust to the same thickness.

※The current value is for one mini-gel. For one wide-gel, set the current value to approximately double.

※When setting 2 or more gels, please doubles the current value of 1 gel by the number of gels.
Ex.：2 times for 2 gels

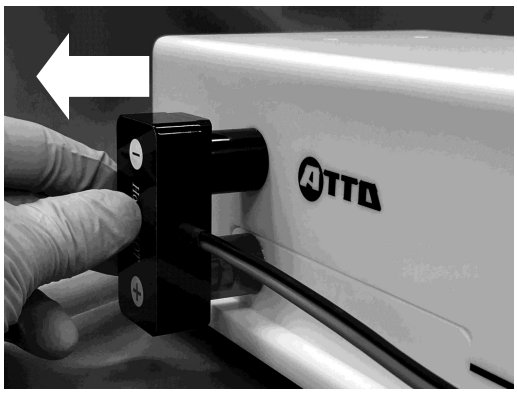
※When using WSE-4055 QBlot kit with 4 mini-gels or 2 wide-gels, please set the voltage value to 12 V.

The optimum conduction time should be determined by preliminary experiment. To maximize the blotting efficiency of the target sample, it is necessary to extend the blotting time. Longer blotting times generally increase blotting efficiency. Please note that when blotting for a long time, the filter paper and the membrane may dry out.

Depending on the power supply (especially high-voltage output type) and setting conditions, the power supply unit may detect an error and fail to due to a sudden increase of current at the start of blotting. If it is caused by the actual output voltage value is small (20~40 V), it may be able to blotting by entering (setting) a number close to the output voltage value. For details, refer to the instruction manual of the power supply.

5.6 After turning off the power

Stop the output of the power supply (or confirm auto-shutdown with a timer), then turn the switch off. Remove the lead assembly with a safety terminal (for R) from the main body and the power supply.



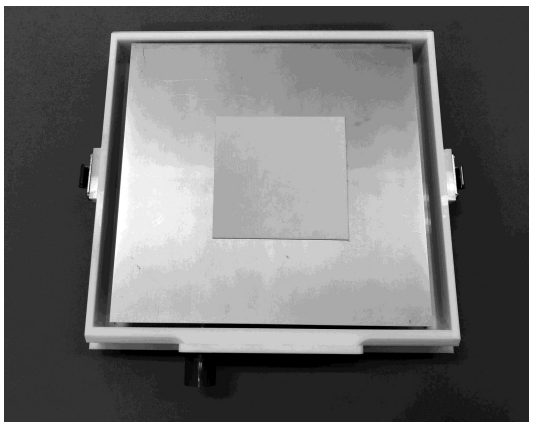
Hold the fixing latches on the left and right sides of the cover and lift the cover upward while pulling up the lever.

※The filter paper and the whole sandwich may stick to the cathode plate. Please pay attention to the fall of the attached filter paper, etc.

Be sure to wear clean experimental gloves on both hands when handling membrane.

Remove the filter paper and gel from the top of the sandwich to collect the membrane. Proceed to the detection of the target molecule through antigen-antibody reactions or staining.

※Filter paper cannot be reused. It may cause uneven blotting and poor blotting efficiency.



5.7 Detection

The following is a rough experimental flow until detection. For details, refer to the instruction manual of the reagent to be used, or references.

1. Blocking

Immediately immerse the blotted membrane into the blocking buffer and shake it for 30 min at room temperature (50 mL/gel).

※ Longer blocking time (over 1 hr) can cause over-blocking. Please be careful.

2. Antibody reaction

1) Dilute the primary antibody with 0.1% Tween-containing EzTBS (TBS-T) or EzPBS (PBS-T) (10 mL/gel).

※ Dilution rate of the antibody varies depending on the antibody. Please refer to the instruction manual attached to each antibody. Generally, it's diluted several hundreds to thousands times.

※ Blocking buffer may be used for antibody dilution.

2) Place the antibody solution of 1) in a container slightly larger than the membrane and react with the membrane while shaking at room temperature for 1 hr.

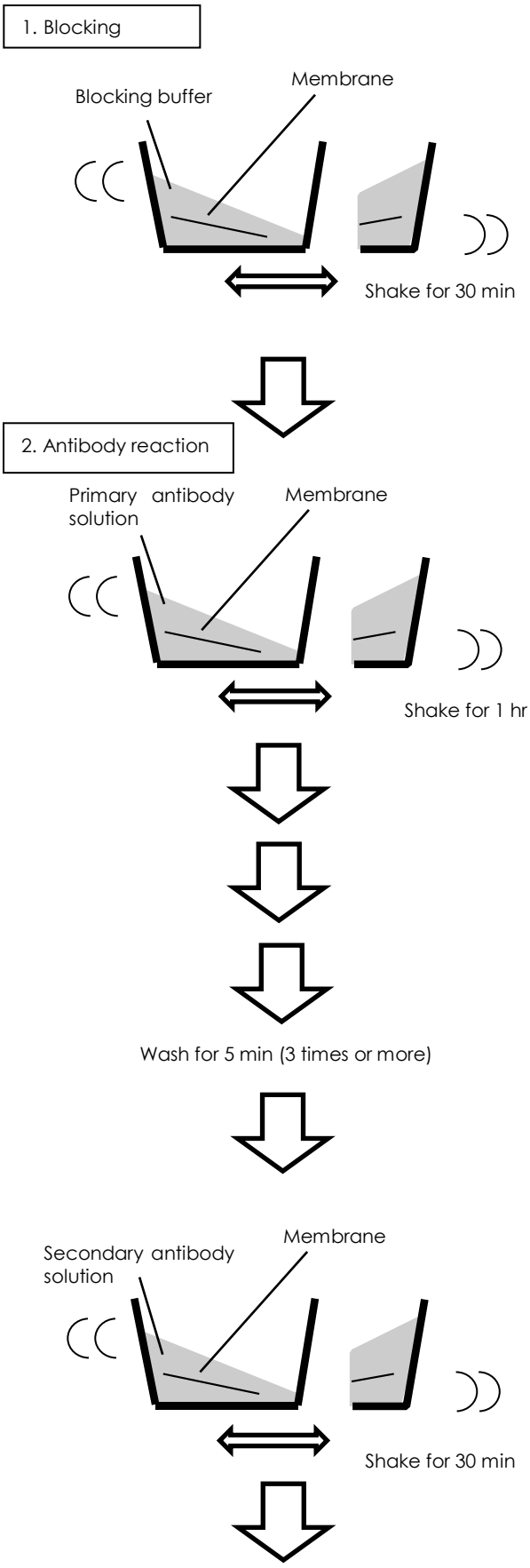
※ Antibody reaction time and temperature vary depending on the antibody. Please refer to the instruction manual attached to each antibody. It usually reacts at room temperature or 37℃ for 30 min~1 hr or 4℃ for overnight.

3) Discard the antibody solution, add 50 mL of TBS-T or PBS-T, and shake for 5 min (washing operation). Washing operation is repeated 3 times or more.

4) Dilute the HRP-labeled secondary antibody with TBS-T or PBS-T (10 mL/gel).

※ Dilution rate of the antibody varies depending on the antibody. Please refer to the instruction manual attached to each antibody. Generally, it's diluted thousands to tens of thousands of times.

※ If the background signal is high, increase the dilution rate of antibodies or add blocking agents to the antibody dilution solution at a normal 1/10 concentration.



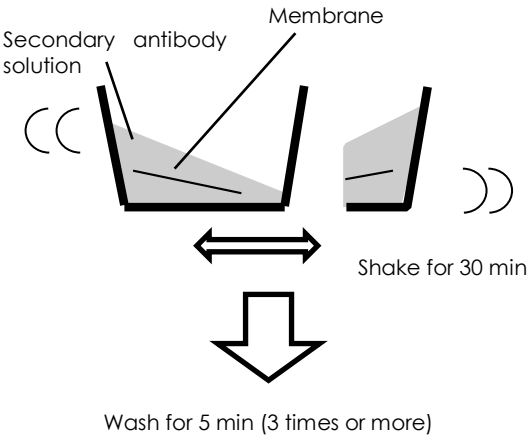
5) Add the antibody solution of 4) to the membrane after washing and react with the membrane while shaking at room temperature for 30 min.

※ Antibody reaction time and temperature vary depending on the antibody. Please refer to the instruction manual attached to each antibody. It usually reacts at room temperature for 30 min to 1 hr, or at 4℃ for overnight.

6) Discard the antibody solution, add 50 mL of TBS-T or PBS-T, and shake for 5 min (washing operation). Washing operation is repeated 3 times or more.

※ Too much washing operation may weaken the signal.

※ If the washing is insufficient, the background signal may become higher.



3. Detection

3. Detection

The following is an example of luminescence detection using the WSE-7110 EzWestLumiOne as a detection reagent.

1) Place the detection reagent in a clean container slightly larger than the membrane.

※ You can use transparent film like 「ATTO Pitatt Clear」 or plastic wrap instead of the container.

2) Immerse the membrane with the detection reagent for several seconds. Please confirm that the detection reagent is immersed the whole membrane.

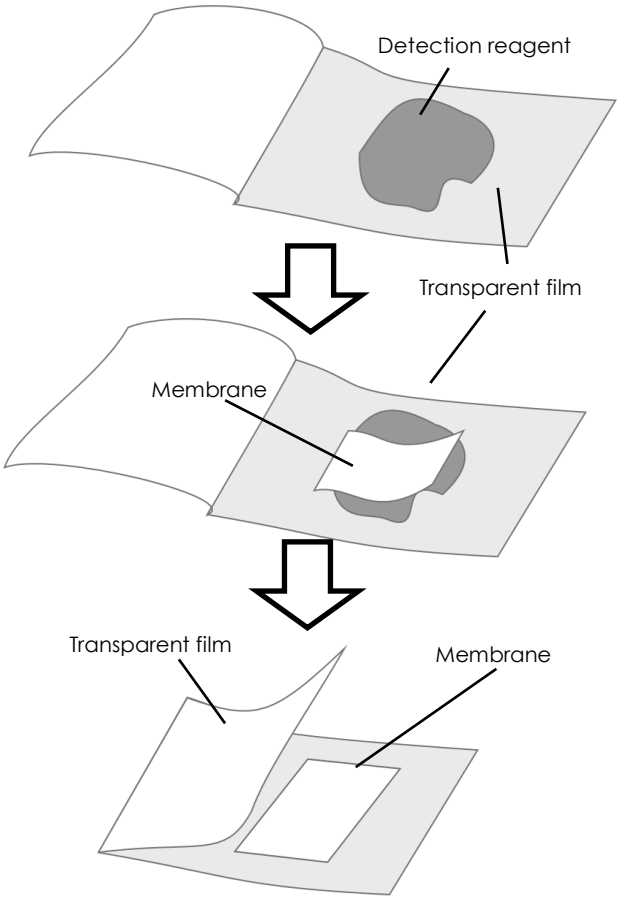
※ If the detection reagent is not uniformly immersed in the membrane, it causes the foamy background and uneven signal.

3) Interlay the membrane in the clear film or plastic wrap, taking care not to trap air bubbles.

※ If there is air between the membrane and the film, it causes the foamy background and uneven signal. Please be careful.

※ When using the plastic wrap, wrinkles of the plastic wrap cause wrinkle-like background and uneven signal. Please be careful.

4) Capture an image of the detection reagent and the membrane with a chemiluminescence imaging system.



ATTO is handling expendable items such as detection reagent (page 25). For details, please download the catalog from our website or ask us. (Please check the back cover.)

In addition, we have「Tip series」ranging from electrophoresis to blotting and detection. For details, please visit and download from our website (https://www.atto.co.jp/eng/technical_info) or QR code below.

1. Application note
Basic operation of Western blotting

2. Application note
Western blotting - Transfer

3. Application note
Western blotting - Blocking

4. Application note
Western blotting - Antibody reaction and detection

5. Operation video (WSE-4110)

5.8 Cleaning and storage of the apparatus

1. Cleaning of the apparatus

Wash the cover and the base under running water before the blotting buffer dries. Electrodes on the cover and the base should be washed using a soft sponge containing neutral detergent and rinsed with purified water. These should be air dried after washing.

A white mark in the shape of a sheet of filter paper remains on the cathode plate, but it should be thoroughly washed using a soft sponge. Immediately after use, it can easily be rubbed off.

※Heavy soiling of the anode and the cathode plate may cause uneven blotting.

※Do not tilt the cathode plate diagonally with pushing one corner of the cathode plate. The internal spring parts may be deformed.

2. Storage of the apparatus

Do not store in places exposed to direct sunlight, high temperature, or where there is a possibility of exposure to corrosive gases.

6 Troubleshooting

For troubleshooting, not only the apparatus but also all operations from electrophoresis to detection such as electrophoresis gel, blotting buffer, detection reagents, etc., need to be confirmed. In addition to the items described in this chapter, please also confirm the instruction manual of the apparatuses and reagents used in each operation.

Symptom	Cause	Solution
No power. Voltage reading on power supply is zero when switched on. Voltage output is stopped while error is displayed on power supply.	Incorrect composition of blotting buffer	Check the composition and preparation of the blotting buffer and re-prepare it if it's different or wrong. (page 9)
	Incomplete connection of safety terminal leads	Completely insert a safety lead assembly with a safety terminal to the power supply until it stops.
	Insufficient pretreatment of the filter paper or membrane	Make sure that filter paper and/or membrane are completely soaked in each blotting buffer during pretreatment. For protein blotting, when a membrane treated with methanol is soaked in blotting buffer, the membrane tends to repel blotting buffer at first, so make sure that the membrane is completely soaked in the blotting buffer by shaking the container slightly strongly.
	Broken wire	Immediately stop the use of this apparatus, and contact us (Please refer to the back cover)
	Filter paper is reused	Filter paper is not reusable. Always use new filter paper.
	Insufficient contact between the cathode and the sandwich	Verify the number of sheets and the thickness of the filter paper to be used. When using other filter paper than our product, use filter paper of 0.9 mm in thickness, or adjust the number of sheets of paper to achieve the same thickness as when our Absorbent Paper is used.
	The gel was soaked in blotting solution for too long	Make sure that the gel before blotting is not soaked in blotting buffer for a long time (more than 5 min). Please start to blotting as soon as possible (page 11).
Cannot see the bands	Blotting is not done correctly	For high molecular weight proteins, it can be improved by prolonging the blotting time and lowering the gel concentration. For low molecular weight proteins, it can be improved by increasing the amount of methanol added to the blotting buffer to about 1.5 to 2 times.
An obscure blotting pattern	Weak contact between the gel and the membrane	After stacking filter paper, membrane and gel, push air bubbles and excessive blotting buffer out by rolling a roller. Excessive blotting buffer between the membrane and the gel may cause the protein to diffuse into the solution and cause an obscure blotting pattern.
Uneven or dirty blotting	Incorrect composition of blotting buffer	Check the composition and preparation of the blotting buffer and re-prepare it if it's different or wrong. (page 9)
	Air bubbles remain in the sandwich	Carefully stack the filter papers, membrane and gel to avoid trapping even small air bubbles in the sandwich (page 13~14).
	Insufficient pretreatment of the filter paper or membrane	Make sure that filter paper and/or membrane are completely soaked in each blotting buffer during pretreatment. For protein blotting, when a membrane treated with methanol is soaked in blotting buffer, the membrane tends to repel blotting buffer at first, so make sure that the membrane is completely soaked in the blotting buffer by shaking the container slightly strongly.
	The sandwich is not placed at the center of the anode plate	Place the sandwich at the center of the anode.

Symptom	Cause	Solution
Uneven or dirty blotting	Filter paper is reused	Filter paper is not reusable. Always use new filter paper.
	The anode or cathode is soiled or substances are adhering to their surfaces	Remove dirt and adhering substances from the surfaces of the anode and cathode by washing them thoroughly. If the surfaces are too dirty, please contact us (Please refer to the back cover).
	Wrong way to stack sheets of filter paper	Verify the order and the number of sheets of filter paper to be stacked. If it is wrong, re-prepare the sandwich by using new sheets of filter paper.
	Insufficient contact between the cathode and the sandwich	Verify the number of sheets and the thickness of the filter paper to be used. When using other filter paper than our product, use filter paper of 0.9 mm in thickness, or adjust the number of sheets of paper to achieve the same thickness as when our Absorbent Paper is used (page 10).
	Problems during the detection step	Contact the manufacturers that produce the reagents used for detection.

7 Maintenance

7.1 Cleaning

1. Cover

Wash the cover under running water before the blotting buffer dries. If the surface is dirty, wash off the surface by soft sponge with a neutral detergent. Electrodes on the cover should be washed using a soft sponge.

A white mark in the shape of a sheet of filter paper remains on the cathode plate, but it should be thoroughly washed using a soft sponge. Immediately after use, it can be rubbed off easily.

Please do not wash with soaking.

If there is dust on electrode plug, please remove it carefully so that the plug won't be scratched.

※Do not tilt the cathode plate diagonally with pushing one corner of the cathode plate. The internal spring parts may be deformed.

Warning Please clean the cover unit after detach from the base unit.

2. Base

Wash the base under running water before the blotting buffer dries. If the surface is dirty, wash off the surface by soft sponge with a neutral detergent. Electrodes on the base should be washed using a soft sponge.

If there is dust on electrode terminal, please remove it carefully so that the connector won't be scratched.

Warning Please clean the base unit after detach from the cover unit.

7.2 Inspection

Please read the instruction manual and inspect it when using it after storage. If there is an abnormality during the inspection, please do not use this apparatus and contact us.

1. Cover

Please make sure that there is no breakage, deformation, or corrosion of the electrode plug. Please confirm with your eyes that the cathode plate is not dirty, that the vertical movement is smooth by pressing the center of the cathode plate, and that there is no difference between the horizontal and vertical movements.

Warning Please check the cover unit after detach from the base unit and the safety lead.

2. Base

Please make sure that there is no breakage, deformation, or corrosion of the electrode terminal.

Please confirm that there is no shaking on the anode plate and that it is firmly fixed.

Warning Please check the base unit after detach from the cover unit and the safety lead.

7.3 Warranty

Please refer to the warranty attached to the product regarding the warranty provision and the warranty period.

7.4 Consumables and reagents

The followings are consumables. Please specify the code number when ordering.

Application		Model	Product Name	Code No.
Blotting membrane	Membrane for protein	WSE-4050	ClearBlot P plus membrane (65×65 mm, 20/pk)	2322450
		WSE-4051	ClearBlot P plus membrane (85×90 mm, 20/pk)	2322451
		WSE-4053	ClearBlot P plus membrane (260 mm×3 m)	2322453
	Pre-wet PVDF membrane and filter paper	WSE-4055	QBlot kit (90 x 85mm)	2322445
Filter paper for blotting	Filter paper for blotting	CB-06A	Absorbent paper (Filter paper) (65×65 mm, 400/pk)	2322437
		CB-09A	Absorbent paper (Filter paper) (85×90 mm, 400/pk)	2392393
		CB-20A	Absorbent paper (Filter paper) (20×20 cm, 100/pk)	2392493
Blotting buffer	Three reagent for semi-dry blotting	AE-1460	EzBlot	2332600
	Transfer buffer For fast semi-dry blotting	AE-1465	EzFastBlot	2332590
	Fast semi-dry transfer buffer for high molecular weight protein	WSE-7210	EzFastBlot HMW	2332595
	Tris-Glycine buffer	WSE-7055	EzRun TG	2332323
Blocking buffer	Non-protein blocking reagent	AE-1475	EzBlock Chemi	2332615
	BSA-containing blocking reagent	AE-1476	EzBlock BSA	2332616
	Casein-containing blocking reagent	AE-1477	EzBlock CAS	2332617
Wash buffer	Tris buffered saline	WSE-7230	EzTBS	2332625
	Phosphate buffered saline (Sterilized)	WSE-7430	EzPBS(-)	2332380
	10% Tween solution	WSE-7235	EzTween	2332626
Detection reagent	TMB substrate for HRP detection (Colorimetric detection)	AE-1490	EzWestBlue	2332630
	Luminescence substrate for HRP (1 bottle type)	WSE-7110	EzWestLumiOne	2332632
	Luminescence substrate for HRP (2 bottle type)	WSE-7120S	EzWestLumi Plus	2332637
		WSE-7120L	EzWestLumi Plus	2332638
Reprobing reagent	Stripping solution	WSE-7240	EzReprobe	2332530
Molecular weight marker	Prestained molecular weight marker	AE-1450	EzStandardPrestainBlue	2332347
	3-color molecular weight marker	WSE-7020	EzProtein Ladder	2332346
Gel・Membrane Sealing bag	Transparent film for Gel・Membrane sealing		Pitatt Clear	2322438

8 Specifications

Product name	HorizeBLOT 4M
Model	WSE-4045
Type	Horizontal semi-dry type
Available number of gel for blotting	4 mini-gels
Electrode size Distance between electrode Polarity	205 mm (W) x 200 mm (D) Minimum: 3 mm, Maximum: 15 mm Cover: Cathode, Base: Anode
Safety function	Lead wire with safety terminal Prevent reverse connection with exclusive leads
Environment of usage	Indoor use only Operating ambient temperature: 5~40℃ Operating ambient humidity: 5~70%RH (No condensation)
Apparatus and status of apparatus	Portable apparatus
Dimensions・Weight Main body	246 mm (W) × 235 mm (D) × 92 mm (H) 3.0kg
Components	Main body Cover unit, Base unit Safety terminal lead, Roller, Instruction manual

9 Contact us

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